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RESEARCH ARTICLE

IN VITRO ANTHELMINTIC ACTIVITY OF AQUEOUS AND ORGANIC EXTRACT OF ROOTS OF PUNICA GRANATUM LINN

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Abstract

Aqueous and organic extracts of *Punica granatum* were evaluated for their anthelmintic activity using Indian adult earthworm called *Pheretima posthuma*. Different concentrations of aqueous and organic extract ranging from 10 to 100 mg/ml were made and tested on said earthworm. These extracts were tested for bioassays which includes time for paralysis and time for death of the worms. Aqueous as well as organic extract exhibited strong anthelmintic activity at concentration of 100 mg/ml. Lower concentrations did not produced significant anthelmintic activity. The standard reference drug which is used for comparing anthelmintic activity of these extracts was piperazine citrate at concentration of 10 mg/ml.

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Introduction:-

Helminthiasis is a worm infestation of humans and other animals even life stock and crops affecting health and food production respectively and has impact on global economic factor (Kumar, 2014). The worms which causes helminthiasis are called as helminths and the drugs which are used for treating helminthiasis are nothing but anthelmintics (Abongwa, 2017). There are various types of worms such as hook worms, fluke worms, round worms, tape worms which causes helminthiasis. The names are given according to their shapes. The major organs which get affected in helminthiasis are stomach and intestine and major symptoms of sever helminthiasis include diarrhea, abdominal pain, general malaise and impaired cognitive development. Chronic helminthiasis by hook worm lead to intestinal bleeding and anemia (Hedley, 2015).

Pheretima is a genus of earthworms. *Pheretima posthuma* are long cylindrical shaped worms having length of 15-30 cm. they are mostly found in moist soil and responsible for vegetables and humus. Their life span is 3 to 10 years (Kutschera, 2010).

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Figure 1:- Earthworm (*Pheretima posthuma*).

Punica granatum Linn (family Punicaceae) commonly known as pomegranate is easily available and widely spread plant in the world. It is widely cultivated throughout Mediterranean region and all tropical regions including temperate, tropical and subtropical regions due to its higher commercial scale (Stover, 2007). Due to its health effects, it is widely used in the universe. According to Ayurveda, as all parts of *Punica granatum* has active chemical constituent, it's all parts has medicinal value. Juice of the pomegranate is used as diuretic activity and cooling effect. It is also used to improve vitamin A level, as antipyretic and mineral supplement (Thangavelu, 2017). Its bark decoction acts against piles bleeding, leaves can be used for threatened abortion, flower buds can be used for nasal bleeding, fruit rind is recommended for gum and teeth bleeding, seed powder is effective in dissolving renal calculi (DeFilipps, 2018). Other beneficial medicinal activities of various parts of *Punica granatum* are immunomodulatory, anti-inflammatory, antidiabetic, anticancer, to treat conjunctivitis etc (Jahromi, 2018).



Figure 2:- Cleaned Pomegranate roots (*Punica granatum*).

Literature review revealed that in Ayurveda it is mentioned that its roots also has various medicinal activities but till now no research work was done on extraction and evaluation of extract of roots of Punica granatum. Hence the said research work is undertaken for determining anthelmintic activity against human adult worm.

Materials and Methods:-

Collection, authentication, drying and extraction of plant material:

The roots of pomegranate were collected in the month of June 2020 from Krushi Vigyan Kendra (KVK), Baramati area. The roots were authenticated from Department of Botany, Shardabai Pawar Mahila Mahavidyalaya, Baramati. For cleaning purpose, dirt and dust from the roots are removed washed with flowing water and dried in shade for one month. After one month bark of the roots is also removed and roots are finely powdered in domestic grinder. Powder is subjected to Soxhlet extraction (continuous hot percolation). For getting aqueous extract double distilled water is used and for getting organic extract petroleum ether was used. After 3 cycles of each, mixture is subjected to rotary evaporation and solvent is recollected. Dried extract is removed by scratching method and collected in clean glass bottle. Dried extract is subjected to various preliminary chemical tests to detecting the presence of active phytochemical/constituents present in it.

Collection of animal and identification:

Pheretima posthuma of 6-9 cm length and weighing about 0.5 to 4 gms were collected at night from burrows of moist soil from farms of Baramati area and were identified from Zoology department, Tuljaram Chaturchnad Science College, Baramati. After identification, they were cleaned using saline solution before their use in activity and then used for anthelmintic activity. The reason behind selecting these worms is that they resembles by anatomy and physiology with intestinal worm parasite of human being.

Drugs and chemicals:

All chemicals used in the study are of analytical pure grade chemicals. 500 mg Avizine tablet from Taj Pharmaceutical Ltd, Mumbai was used for preparing standard piperazine citrate solution. Tablet was dissolved in 50 ml double distilled water to get concentration of 10 mg/ml.

Anthelmintic activity:-

The activity was carried out by using the method reported by Ajaiyeoba (Ajaiyeoba, 2008). Necessary changes were done in method as per requirement. 20 ml formulation containing three different concentrations of 10, 50 and 100 mg/ml of aqueous and organic extract were prepared and transferred in 9 different petri dishes. Six *Pheretima posthuma* worms of approximately same size and same length were placed in these petri dishes. Time for paralysis i.e. duration in which movement of worms stop is noted when petri dishes are shaken vigorously and also time of death i.e. period required for death worms is also noted which is confirmed by shaking petri dishes vigorously as well as by dipping petri dishes in hot water for few minutes. Piperazine citrate was used in concentration of 10 mg/ml as reference standard and distilled water was used as control. Results are expressed in mean \pm SEM of six worms in each group and are tabulated and graphically represented.

Table 1:- Time taken for paralysis and death in minutes.

Test substance	Conc. (mg/ml)	Paralysis time (Min)	Death time (Min)
Distilled water	-	-	-
Aqueous extract-I	10	24 \pm 0.4	64 \pm 0.3
Aqueous extract-II	50	17 \pm 0.6	41 \pm 0.8
Aqueous extract-III	100	11 \pm 0.3	27 \pm 0.5
Organic extract-I	10	25 \pm 0.7	67 \pm 0.8
Organic extract-II	50	17 \pm 0.8	46 \pm 0.7
Organic extract-III	100	9 \pm 0.9	29 \pm 0.1
Piperazine citrate	10	22 \pm 0.2	60 \pm 0.3

Results and Discussion:-

Aqueous and organic extract of roots of Punica granatum showed the potent anthelmintic activity. These extracts took the least time for causing paralysis and death of the earthworms. As shown in Table no. 1, aqueous and organic

extract showed essential anthelmintic properties with 100mg/ml giving a shortest time of paralysis in earthworm 11 ± 0.3 and 9 ± 0.9 respectively.

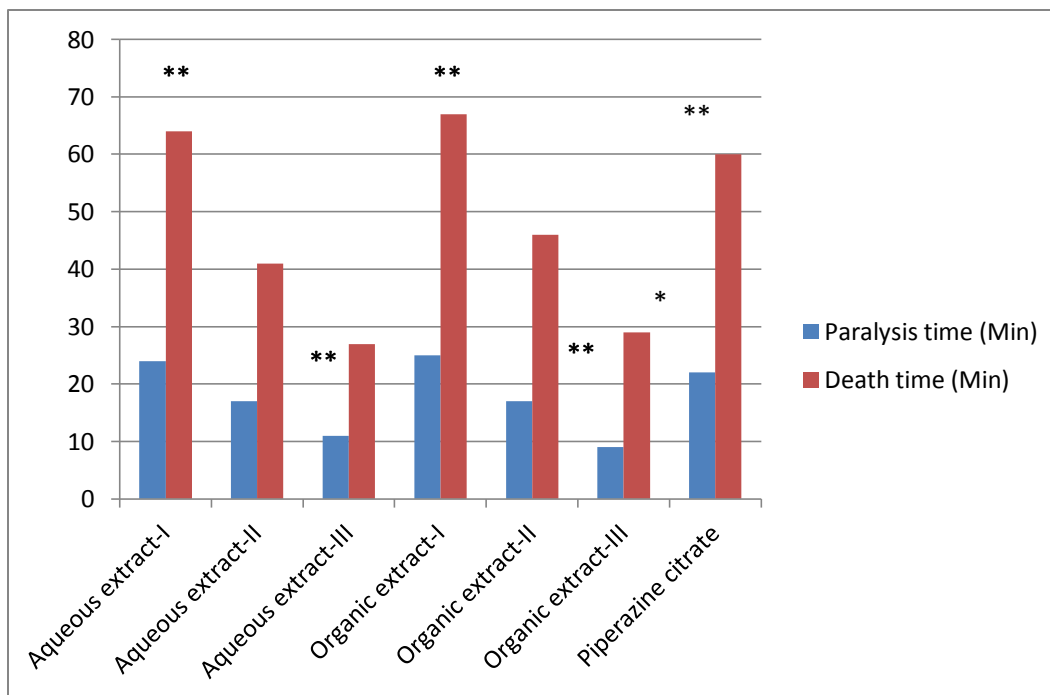


Figure 2:- Paralysis time and Death time in minutes.

Values represent mean \pm SEM, One way ANOVA, $P < 0.0001$, followed by Dennett's t-test, $**P < 0.01$ Organic extract have more effective as that of aqueous extract for paralyzing the earthworm. Aqueous and organic extract showed necessary anthelmintic properties with 100 mg/ml giving a shortest time of Death in earthworm 27 ± 0.5 and 29 ± 0.1 respectively. Aqueous extract have more effective as that of organic extract for time taken to the death of earthworms. The function of the anthelmintic drugs like piperazine citrate acts by paralyzing the worms so that they are excluded in the faeces of human and animals. The extracts not only confirmed this property but also caused death of the worms, especially at 100 mg/ml dose when compared with standard drug piperazine citrate both Aqueous and organic extract of roots of *Punica granatum* showed significant anthelmintic activity at 100 mg/ml dose. In conclusion, the roots of plant *Punica granatum* have been confirmed to exhibit anthelmintic activities.

Future scope:-

From the results it is evident that roots of pomegranate shows potential anthelmintic potential. Hence detecting active phytochemical constituents responsible for the activity by means of chemical analysis and determining their pharmacological mechanism of action is a future challenge. Secondly development of herbal formulation of roots of pomegranate and its characterization by pharmaceutical parameters can be a future scope.

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